

Changes in nitrogen content and isotopic composition in subarctic *Empetrum nigrum* seeds in the period 1976–2010

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High latitude ecosystems are believed to be sensitive to increases in the availability of nitrogen (N) which may be the result of anthropogenic emissions or may be due to climate warming which accelerates the release of N from organic matter in the soil. These changes may be detected in the changes in N pools and their isotopic composition. We studied N content (N%) and isotopic composition ($\delta^{15}\text{N}\text{‰}$) along an elevational gradient in a subarctic environment in northern Finland, using *Empetrum nigrum* (crowberry) seeds collected annually over the past 35 years. We studied whether N% or $\delta^{15}\text{N}\text{‰}$ shows trends that can be attributed to anthropogenic nitrogen, and investigated the effect of climate variables on the N content of seeds. Among the years, variations in N% and $\delta^{15}\text{N}\text{‰}$, were mainly dependent on previous summer or growing season temperatures and were in opposite directions for N% and $\delta^{15}\text{N}\text{‰}$. Among the sites, N% and $\delta^{15}\text{N}\text{‰}$ were highest at a low elevation site, and lowest at the highest elevation site. Trends in $\delta^{15}\text{N}\text{‰}$ over time were contrasting among the sites: at the low elevation site trend was increasing, whereas at the highest elevation site the trend was decreasing. The trend in N% over time was increasing at the low elevation site. Our results suggest that N cycling in these systems has changed in the past 35 years, but there were differences among sites.

Introduction

In the arctic and subarctic environments where inorganic nitrogen availability is generally very low and where plant communities have developed under conditions of nitrogen (N) limitation, increases in N can have substantial effects

on the ecosystems. It is generally believed that nutrient availability to plants in these ecosystems is controlled mainly by the processes involved in soil organic matter decomposition (Robinson 2002). These processes are strongly regulated by soil temperatures. It is thus expected that current climate warming will increase the mineraliza-

tion of nitrogen and other nutrients, enhancing the nutrient availability to plants (Rustad *et al.* 2001). Also atmospheric nitrogen deposition from anthropogenic sources is a continuing problem, and although emissions have declined over past decades, it is difficult to predict the future development of these emissions (Reis *et al.* 2009).

The predicted increases in N availability in the arctic may lead to latitudinal and altitudinal advances of vegetation cover and change the vegetation types. The vegetation change will lead to increased litter input and change in litter quality. This is likely to change carbon dynamics (Jackson *et al.* 2002). Further, landscape changes in the arctic regions may change surface energy budget by reducing albedo and increasing solar energy absorption, thus amplifying the rate of climate warming (Chapin *et al.* 2005). Walker *et al.* (2006) considered that vegetation at high latitudes may lose biodiversity, as the more rapid growth of fast growing species will enable them to dominate the slow growing alpine and arctic species.

Changes over time in the N cycle can be detected in changes of N pools and their isotopic composition. The N isotopic composition ($^{15}\text{N}/^{14}\text{N}$) is commonly denoted as $\delta^{15}\text{N}$ with negative or positive values indicating whether the sample is depleted or enriched, respectively, in the heavier isotope (*see* Material and methods). Soil contains N pools that differ in their isotopic composition. Isotopic composition of a soil N pool depends on the source of N to the soil and is modified by several processes in the soil including isotopic fractionation in N transformations (Amundson *et al.* 2003). Also mycorrhizal fungi alter $\delta^{15}\text{N}$ values in soil when they supply N to plants (Hobbie and Högberg 2012). In addition, N pools have been found to have a vertical pattern in ^{15}N composition in soil profile (Garten 1993). Plant $\delta^{15}\text{N}$ values are affected by the isotopic composition of source N pool and by the presence and the type of mycorrhizal association in the plants roots. Non-mycorrhizal plants have the highest $\delta^{15}\text{N}$ signature, arbuscular mycorrhizal and ectomycorrhizal lower and ericoidmycorrhizal the lowest, 3%–8‰ lower than non-mycorrhizal plants (Michelsen *et al.* 1998, Craine *et al.* 2009).

The change in plant $\delta^{15}\text{N}$ signatures can reflect the N availability in the ecosystem. Studies have commonly found increased $\delta^{15}\text{N}$ with increasing N availability (Cook 2001, Craine *et al.* 2009) or decreased $\delta^{15}\text{N}$ with decreasing N availability (Garten *et al.* 2011). There are two potential reasons for this. In environments where N availability is high, N is lost from the system, and the lost N is more likely to be depleted in the heavier isotope, which increases the $\delta^{15}\text{N}$ value of N remaining in the soil. The other reason is that, when N availability is low plants are more likely to depend on mycorrhizas for N acquisition (Hobbie and Colpaert 2003, Hobbie and Hobbie 2008). The $\delta^{15}\text{N}$ signature in the compounds that are transferred to the host plants of mycorrhizas are depleted in ^{15}N thus decreasing the plant $\delta^{15}\text{N}$ value (Hobbie and Hobbie 2008).

The plant isotopic composition will also reflect the isotopic composition of N from anthropogenic N deposition in cases when isotopic composition of N sources being deposited to the soil differ from the composition of N naturally present in soils (Pearson *et al.* 2000). Depositional N from industrial sources, transport and agriculture can vary both according to the source and, within the same source, temporally due to development of new processes, techniques and instrumentation. The proximity of an N source also affects the vegetation $\delta^{15}\text{N}$ value. The further a plant grows from the point source the closer its $\delta^{15}\text{N}$ value is to the background value. $\delta^{15}\text{N}$ emitted from point sources show large fluctuations. Widory (2007) measured nitrogen oxides (NO_x) emitted from vehicles using diesel or unleaded gasoline to be around 4.6‰ in $\delta^{15}\text{N}$; Ammann *et al.* (1999) obtained a mean value of 5.7‰ near a highway in Switzerland; Heaton (1990) measured negative values from vehicle exhaust and values ranging from +6‰ to +13‰ in $\delta^{15}\text{N}$ from coal-fired boilers. Widory (2007) compared central-heating sources using different types of combustible and found a value of –5.3‰ for coal, $-7.5\text{‰} \pm 8.3\text{‰}$ for fuel oil and values ranging from 2.9% to 15.4% for natural gas.

On a global scale, $\delta^{15}\text{N}$ has been found to decline during the last century. Information obtained from herbarium stored plant samples from Mediterranean (Peñuelas and Estiarte 1997), and Europe (Solga *et al.* 2006), lake sediment

Fig. 1. Typical plant community of the Värrio region with *Empetrum nigrum*, *Vaccinium vitis-idaea* and *Cladonia rangiferina*.



in the Arctic (Wolfe *et al.* 2006), as well as ice cores (Freyer *et al.* 1996, Hastings *et al.* 2009) all show a consistent decreasing trend. The observed trend coincides with the period of increased fossil fuel emissions (Hastings *et al.* 2009); however, the values and rate of change vary among areas.

Here we aimed to study whether anthropogenic N deposition had had an effect on the N availability along an elevational vegetation gradient in northern Finland, by utilizing a unique 35-year-long annual-resolution time series of crowberry berries and their seeds that were systematically collected from fixed sample plots around the Värriö Subarctic Research Station. It is likely that variations in wind speed and temperature along the elevation gradient will affect the availability of nitrogen, as wind speed can affect the deposition rate, and temperature is likely to influence the mineralization rate. Moreover, changes in N emissions over 35 years certainly occurred (Hole *et al.* 2009, Forsius *et al.* 2010) and this may be evident in the data if the atmospheric source of N dominates the soil source.

The crowberry (*Empetrum nigrum*; Fig. 1) is an evergreen dwarf shrub with an almost circumpolar distribution across the northern parts of the northern hemisphere. It is important as vegetation cover in many northern high-latitude ecosystems and an important constituent of the understorey vegetation in the boreal forest in Fennoscandia (Tybirk *et al.* 2000). It forms both

ericoid mycorrhizal and dark-septate endophyte associations (Ruotsalainen *et al.* 2010). It has been suggested that the crowberry, as many ericoid plants, relies strongly on organic N provided by the mycorrhiza, thus giving it an advantage in competition on nutrient poor habitats (Tybirk *et al.* 2000).

The study area in northern Finland is remote and generally considered unpolluted, although SO_2 , CO, NO_x , hydrocarbons, dust particles and trace metals from the nickel-copper smelters of Nikel and Montchegorsk in northwestern Russia may potentially be transported in the atmosphere to the Finnish side (Ruuskanen 2003). We measured N and $\delta^{15}\text{N}$ from berry seeds. We hypothesized that if changes in N availability had occurred at our study site, either from increased mineralization or from atmospheric deposition, it should be seen in the combined measurements of N and $\delta^{15}\text{N}$ time series. That is because N% does not necessarily change due to increased N availability since it can be used for increased growth. $\delta^{15}\text{N}$, instead, is an indicator of source N and soil processes. The relatively long time series also provide a good opportunity to study the climatic and environmental effects on the crowberry N budget and its relationship to berry production.

Material and methods

The berry samples were collected close to the

Värriö Subarctic Research Station (67.748°N, 29.610°E). The station is located in the Värriö Strict Nature Reserve in northeastern Finland, less than 10 km from the Russian border. The area belongs to the northern boreal zone (Ahti *et al.* 1968) and is topographically characterised by fells with gentle slopes, with highest treeless summits reaching 600 m above sea level (a.s.l.). The soils are stony, and the podzolised undifferentiated tills that overlay the serpentine-origin bedrock gradually decrease in thickness with elevation, so that the summits consist typically of rocks covered with a thin organic layer. The distance from the nearest small road is 8 km and there are no towns or industry nearby. The nearest point sources of pollution are mining industry and smelters on the Russian side, Montchegorsk located 150 km to the east and Nikel located 190 km to the north of the reserve. The prevailing wind direction in the autumn and winter is south-west. In spring and summer, the winds are more equally distributed (Ruuskanen *et al.* 2003).

The berries were collected from four 10 × 10 m sample plots, located at different elevations. Site 1 is located on the top of the Värriö I fell at 470 m a.s.l. The vegetation is fell field vegetation, consisting of lichens and dwarf shrubs such as *Empetrum nigrum*, *Arctostaphylos alpina*, *Vaccinium vitis-idaea*. Site 2 is in the mountain birch zone on the northern slope of the Värriö I fell at 415 m a.s.l. The vegetation at this elevation consists of mountain birch (*Betula pubescens* subsp. *czerepanovii*), dwarf shrubs (e.g. *E. nigrum*, *Vaccinium uliginosum*, *Vaccinium myrtillus*), and scattered Scots pines (*Pinus sylvestris*). Site 3 is located at the northern side of the fell at 360 m a.s.l. The open-canopy forest at this elevation consists of a mixture of the Norway spruce (*Picea abies*) and Scots pine. The ground vegetation consists of mosses, shrubs and forbs, (e.g. *E. nigrum*, *V. uliginosum*, *V. myrtillus*, *Rubus chamaemorus*). Site 4 is located on the nearby Kotovaara hill at 370 m a.s.l. The site is Scots pine-dominated forest, with a ground vegetation consisting of graminoids and shrubs (e.g. *E. nigrum*, *V. myrtillus*, *V. vitis-idaea*). Tree stand structure can be assumed to have remained relatively unchanged in the three first areas on the slopes of the Värriö I fell, with absent or only sparse canopy. However, judged from his-

torical aerial photographs, the stand density and canopy cover at site 4 increased in the past. This most likely occurred as a response to the drastic reduction in surface fires that were common in the region prior to the 20th century (Wallenius *et al.* 2010).

At each site, the crowberries have been collected every year since 1976. The date when the berries were picked varied between 3 August and 27 September. All the berries from the plot were picked on each occasion. The berries were dried in an oven at 70 °C for approximately 24 hours, and stirred with a spoon during drying. The weight of the berries was measured before and after drying. Berries from the year 1990 appeared “burned” and differed considerably in their dry mass from those of other years, so we decided to exclude this year from the analyses.

Seeds, not berries, were analysed for N to avoid possible contamination from outside sources, to reduce possible effects from variations in the degree of drying and since the seed material was better suited for grinding. For the N content and isotope analysis approximately 15–20 berries were taken. The berries were put in distilled water in a beaker and broken with tweezers. The seeds from inside the berries dropped on the bottom of the beaker and were collected from there. Care was taken to include as little berry flesh as possible in the sample. The seeds were then dried in an oven at 50 °C for approximately 48 hours, and then ground to a powder with a ball mill.

The samples were weighed (approximately 2.4 mg) and packed in tin capsules and measured in the Laboratory of Chronology at the University of Helsinki with an isotope ratio mass spectrometer (DeltaPlus Advantage, Thermo Scientific, Bremen, Germany) connected on-line via an interface (ConFlo III; Thermo Scientific) to an elemental analyzer NC 2500 (CE Instruments, Milan, Italy). Always at least two subsamples were measured and the result was calculated as their average. Isotopes are expressed as delta (δ) values: $\delta^{15}\text{N}\text{‰} = (R_{\text{sample}} - R_{\text{stand}})/R_{\text{stand}} \times 1000$, where R_{sample} represents the sample isotope ratio, and R_{stand} the ratio of atmospheric N_2 . Accuracy, determined by comparing measured value to the known value of a laboratory standard, was 0.2‰ for $\delta^{15}\text{N}$. Precision for $\delta^{15}\text{N}$, within the analyzed

samples, estimated as standard deviation (SD) of repeated measurements was on average 0.2‰. The nitrogen percentages were derived from the same measurements. Accuracy for N% was estimated to be 0.2%, by comparing measured values to laboratory standards. Precision of the measurement, the SD between repeated measurements in the same batch was estimated to be 0.02% on average for N%.

The weather data we used were collected routinely by the Finnish Meteorological Institute (FMI) at the Värriö research station. Of the weather variables, sunshine hours were not available from the station and thus we used the data from Sodankylä (measured FMI), 130 km to west from our study area. The mean annual temperature for the studied period is $-0.6\text{ }^{\circ}\text{C}$ and it exhibits a significant (Mann-Kendall test: $Z = 3.52$, $n = 35$, $p < 0.001$) increasing trend of $0.05\text{ }^{\circ}\text{C}/\text{year}$ (non-parametric Sen's method $Q = 0.053$) over the 35-year period. Annual precipitation sum is on average 589 mm. Winter and spring precipitation increased significantly (Mann-Kendall test: $Z = 2.46$, $n = 35$, $p = 0.018$; and $Z = 2.03$, $n = 35$, $p = 0.050$; respectively). Snow cover in the area usually lasts from late October to mid May.

Trends in $\delta^{15}\text{N}\text{‰}$, N% and berry yield series were tested using the Mann-Kendall test. For testing the influence of climate on $\delta^{15}\text{N}\text{‰}$, N% and berry yield, the time series were averaged from our four sites. As the measure of berry yield, dry weight was used, because we noticed that the fresh weight was consistently higher and the dry weight percentage smaller the later in autumn the berries were picked. The averaged series followed normal or nearly normal distribution. The series of $\delta^{15}\text{N}\text{‰}$, N% and dry weights were correlated (Pearson's correlation, r_p) with monthly averages of temperature and precipitation from previous and current year, with seasonal averages, and with annual precipitation, i.e., the precipitation sum from previous September to current August representing the "hydrological year". The correlations were calculated with MS Excel. To investigate the factors that explain the variation in berry $\delta^{15}\text{N}\text{‰}$, N% and dry weight, multiple linear regressions were computed. As possible explanatory variables, we included seasonal averages of tem-

perature, precipitation and sunshine hours from previous and current year (from previous spring to current summer). We also included average of sunshine hours from May to June from previous and current year, because of their possible effect on success of pollination. Average temperature from May to September from previous and current year was included as a variable that averages the snowless season temperatures. In addition, timing of snowmelt (days since the beginning of the year when the permanent snow cover disappeared), as well as snow depth in the period from November to April measured along a line across the Värriö I fell, both from previous and current year, were included. Stepwise multiple linear regressions and related statistics were calculated with R version 2.13.1 (R Development Core Team 2011). Akaike's Information criterion (AIC) was used to identify the best combination of explanatory variables to be included in the multiple linear regressions model. The candidate model with the minimum AIC value was selected.

Results

$\delta^{15}\text{N}$, N% and berry yield

The $\delta^{15}\text{N}$ values measured from the seeds ranged between -7.0‰ and 0‰ (Fig. 2). Lowest values were measured for site 1 on top of the Värriö I fell, and the highest values were for site 4 in the forest (site: 1 -5.5‰ , site 2: -5.5‰ , site 3: -4.1‰ , site 4: -1.7‰). At site 4, where the $\delta^{15}\text{N}$ values were high, $\delta^{15}\text{N}$ values also increased significantly (Mann-Kendall test: $n = 34$, $Z = 4.95$, $p < 0.001$) during the period studied. The values of $\delta^{15}\text{N}$ at site 1 were decreasing (Mann-Kendall test: $Z = -3.62$, $n = 34$, $p < 0.001$).

The measured N% ranged between 0.3% and 1.1% (Fig. 3). Again, lowest values were measured for sample plots on the fell and highest for the site in the forest (site 1: 0.65%, site 2: 0.63%, site 3: 0.76%, site 4: 0.88%). Only at site 4, the N% values increased during the study period (Mann-Kendall test: $Z = 2.52$, $n = 34$, $p = 0.016$).

The yield of berries varied greatly among the years but there was no general trend over the 35-year period (Fig. 4). The annual variation was

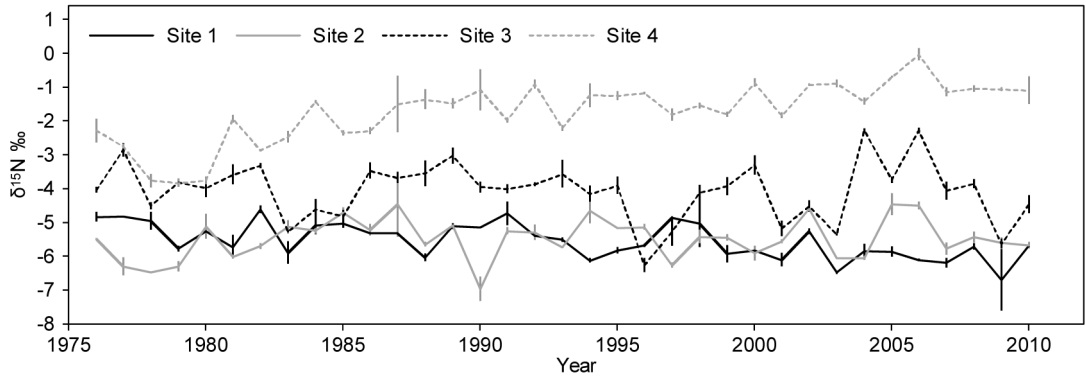


Fig. 2. $\delta^{15}\text{N}$ values of seeds. Site 1 (black) on top of the Värriö I fell, site 2 (grey) in the mountain birch zone, site 3 (dashed black) on the bottom of the Värriö I fell, and site 4 (dashed grey) on Kotovaara. Error bars indicate ± 1 SD, calculated from 2 to 6 measurements.

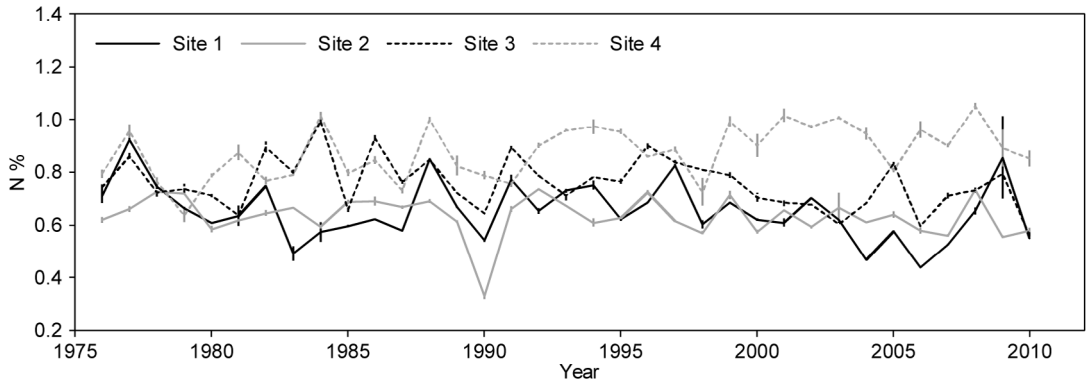


Fig. 3. Nitrogen percentages of seeds. Site 1 on top of the Värriö I fell, site 2 in the mountain birch zone, site 3 on the bottom of the Värriö I fell, and site 4 on Kotovaara. Error bars indicate ± 1 SD, calculated from 2 to 6 measurements.

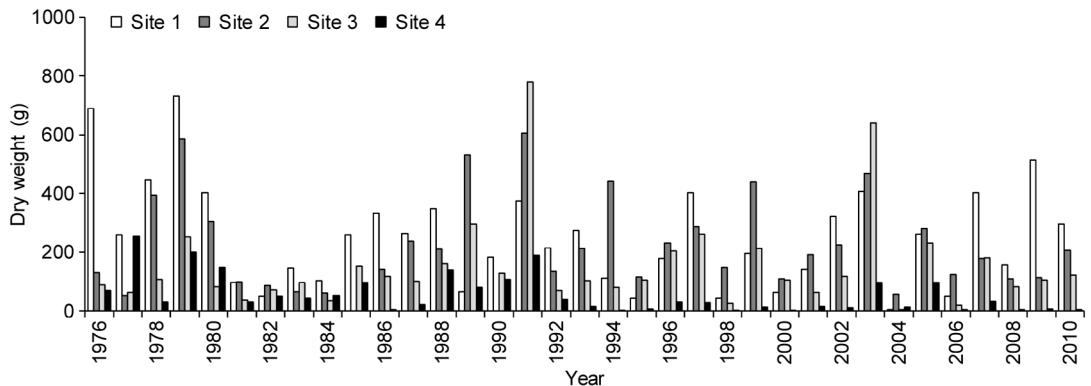


Fig. 4. An annual time series of the total dry weight of crowberry berries for each 100 m² plot.

similar among sites: all sites correlated significantly with each other. The weakest correlation was found between sites 1 and 3 ($r_p = 0.33$, $p = 0.05$). Site 1, on the top of the fell, was the most productive, the dry weight being on average

252.5 g. Sites 2, 3 and 4 produced on average 229.9 g, 151.8 g and 56.0 g per year, respectively. Despite a general lack of trend, there was a trend towards lower yields at site 4 (Mann-Kendall test: $Z = -3.22$, $n = 34$, $p = 0.003$).

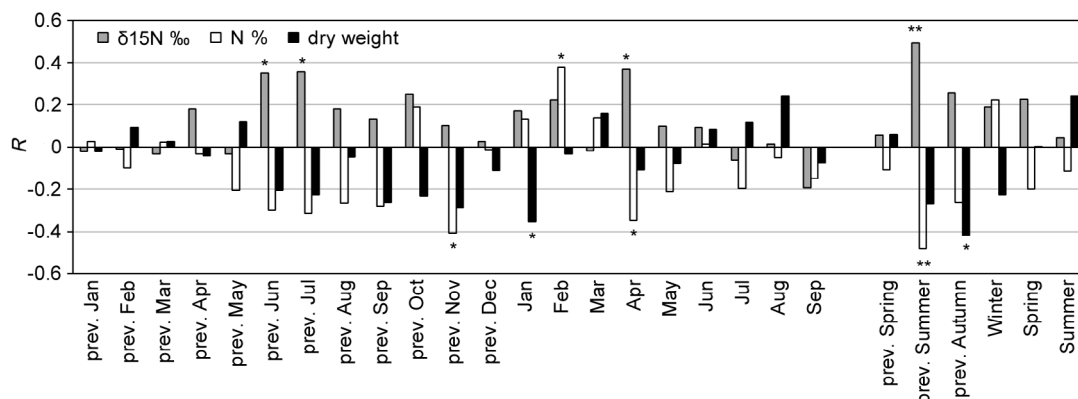


Fig. 5. Correlations between averaged $\delta^{15}\text{N}\text{‰}$ series, averaged N% series, averaged dry weights of berries and monthly temperature. Asterisks denote statistical significance of correlations (* $p < 0.05$ and ** $p < 0.01$). Spring = March, April, May. Summer = June, July, August. Autumn = September, October, November. Winter = December, January, February.

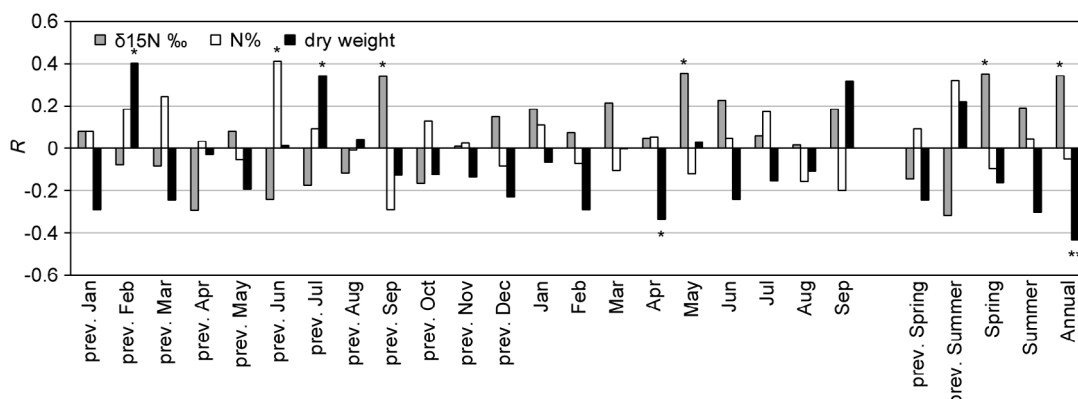


Fig. 6. Correlation between averaged $\delta^{15}\text{N}\text{‰}$ series, averaged N% series, averaged dry weight of berries and monthly precipitation amount. Asterisks denote statistical significance of correlations (* $p < 0.05$ and ** $p < 0.01$). Spring = March, April, May. Summer = June, July, August, Annual = from previous September to current August.

Averaged $\delta^{15}\text{N}\text{‰}$ and N% series did not correlate with each other. In addition, there was no systematic correlation between $\delta^{15}\text{N}\text{‰}$ and N% series at the sites. Average dry weight of the berries correlated negatively with $\delta^{15}\text{N}\text{‰}$ ($r_p = -0.36$, $p = 0.032$). Dry weight of the berries did not correlate with N%. The day of year when berries were picked did not correlate with dry weight of the berries or seed $\delta^{15}\text{N}\text{‰}$ or N%. In addition, we studied the correlations for lagging years to see if, for example, high N content in one year could affect the berry production in the next. However, we obtained only one weak correlation within 4 years: N% two years previously correlated negatively with $\delta^{15}\text{N}\text{‰}$ ($r_p = -0.36$, $p = 0.037$).

Climatic correlations

Averaged $\delta^{15}\text{N}\text{‰}$ series correlated most strongly with previous summer temperatures (Fig. 5). $\delta^{15}\text{N}\text{‰}$ also correlated with April temperature and with spring precipitation, previous September precipitation and annual precipitation (Fig. 6). Averaged N% series had systematically negative correlation coefficients with temperature. N% correlated negatively and most strongly with previous summer temperature. It also correlated negatively with previous November and April temperature and positively with February temperature and with previous June precipitation. Dry weight of the berries correlated negatively and most strongly with annual

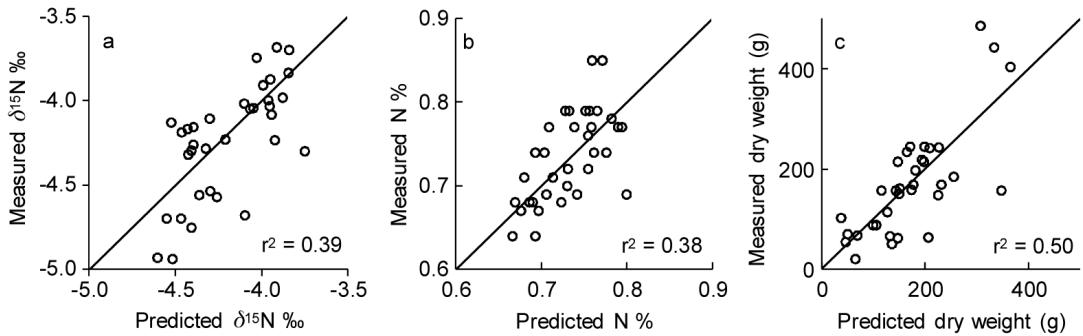


Fig. 7. Relationships between measured and predicted (a) $\delta^{15}\text{N}\text{‰}$, (b) $\text{N}\%$, and (c) dry weight of berries. The equations are given in Table 1.

precipitation. It correlated positively with previous February and previous July precipitation and negatively with April precipitation and January temperature.

$\delta^{15}\text{N}\text{‰}$ was predicted with a multiple linear regression that used previous summer precipitation, timing of previous year snow melt, previous summer sunshine hours, spring precipitation and previous summer temperature as explanatory variables (Fig. 7 and Table 1). $\text{N}\%$ was predicted with the previous year's May to September temperature and winter temperature. Dry weight of berries was best predicted with a combination of previous autumn temperature, previous year snow depth, annual precipitation sum, previous year spring sunshine hours and previous year May to September temperature. In general, the previous-year environmental conditions seemed more important for the berries than current-year ones. However, the models were complex and the predictions explained 50% of the variance in dry weight of the berries, 38% of the variance in $\text{N}\%$, and 39% of the variance in $\delta^{15}\text{N}\text{‰}$, meaning that the explaining power of the models was quite low.

Discussion

The environment under study, the subarctic of northern Finland, is sensitive to human-induced changes in the environmental conditions. Despite the remoteness of the area, increases in plant-available nitrogen are expected to have occurred due to human activity (Hole *et al.* 2009, Forsius *et al.* 2010) or due to increased mineralization brought about by raised soil temperatures during

the last 35 year period (Helama *et al.* 2011). The lack of a general trend during this period, in yield or $\text{N}\%$, suggests that any such effect is too small to be detectable in berry harvests.

Between different environments, increases in leaf nitrogen concentration are usually accompanied by increases in leaf ^{15}N values. Foliar ^{15}N and foliar $\text{N}\%$ have been found to increase with mean annual temperatures and decrease with mean annual precipitation (Craine *et al.* 2009). At our study sites, both $\delta^{15}\text{N}\text{‰}$ and $\text{N}\%$ were higher at the forested sites than higher up the fell, with lower growing season temperatures. Our results are also consistent with those of Michelsen *et al.* (1998) who found lower values of $\delta^{15}\text{N}\text{‰}$ and lower nitrogen concentrations in leaves of shrubs (including *Empetrum*) from heath tundra than in those from forest tundra in Sweden, and consistent with findings from the Alps (Huber *et al.* 2007).

Any variation in the time series due to nitrogen deposition can be caused either by increasing availability of N that increases $\delta^{15}\text{N}\text{‰}$ and $\text{N}\%$, or by a change in source N that can shift the $\delta^{15}\text{N}\text{‰}$ value to either direction. We detected two statistically significant trends in our $\delta^{15}\text{N}\text{‰}$ time series. The trend found for site 4 is most likely due to an increased nitrogen availability. At this site, there was an increase in the N concentration in the seeds and a decrease in the yield of berries. In a similar environment, *Empetrum* has been found to respond to N addition by decreasing berry yield (Shevtsova and Neuvonen 1997). Why should this site behave differently from the others? At this site, the immobilized N reserves of the soil are probably largest due to thicker organic

soil layer, and so the effect of temperature on mineralization might have a large absolute effect on the supply of N to the roots. Moreover, this site faces southeast exposing it to southerly and easterly winds, whereas the other sites are on the northern slope of Värriö I or on the top of the fell. In addition, this site is also closest to the research station (200 m) and we cannot completely rule out the possibility that the proximity of the station could cause N fertilization from human excreta. Judged from historical aerial photographs, stand density and canopy cover have increased in the area around this site, which could, in addition to increased N, explain the decreased berry yields observed on this site. Our results show that the berry yields tend to be higher at open sites.

The other significant trend was in the opposite direction and was found for site 1, at the highest elevation. At this site, the rates of turnover and supply of soil N are expected to be lower due to the effect of altitude on temperature, and this could cause depositional N to have a proportionately greater effect on the N pool. Ruuskanen *et al.* (2003) measured the highest NO_x concentrations in Värriö during easterly winds, suggesting transport from industrial areas in Kola, namely Montchegorsk. However, especially in winter and spring also elevated concentrations of NO_x were measured during southerly winds, indicating that NO_x could originate from anthropogenic sources further away. This is significant since the prevailing wind direction is from south-

Table 1. Multiple regression models. $T_{\text{prevAutumn}}$ = previous autumn temperature ($^{\circ}\text{C}$), SND_{prev} = previous year snow depth (cm), P_{annual} = annual precipitation (mm), $S_{\text{prevSpring}}$ = previous spring sunshine hours (h), $T_{\text{prevMaySep}}$ = previous May to September temperature, T_{winter} = winter temperature, $P_{\text{prevSummer}}$ = previous summer precipitation, SNM_{prev} = previous year timing of snowmelt (day of year), $S_{\text{prevSummer}}$ = previous summer sunshine hours, P_{spring} = spring precipitation, $T_{\text{prevSummer}}$ = previous summer temperature. Seasons as in Figs. 5 and 6.

	<i>F</i>	df	<i>p</i>	Adjusted <i>R</i> ²	AIC
Model for dry weight	7.589	28	0.00013	0.50	300.64
Multiple regression terms	Estimate	SE	<i>t</i>	<i>p</i>	
$T_{\text{prevAutumn}}$	−34.001	10.879	−3.125	0.004	308.81
SND_{prev}	−4.756	1.494	−3.184	0.004	309.14
P_{annual}	−0.433	0.157	−2.767	0.010	306.85
$S_{\text{prevSpring}}$	45.200	14.932	3.027	0.005	308.26
$T_{\text{prevMaySep}}$	−28.717	18.249	−1.574	0.127	301.52
	<i>F</i>	df	<i>p</i>	Adjusted <i>R</i> ²	
Model for $\text{N}\%$	11.35	32	0.0002	0.38	−211.07
Multiple regression terms	Estimate	SE	<i>t</i>	<i>p</i>	
$T_{\text{prevMaySep}}$	−0.0448	0.0097	−4.599	0.0001	−209.78
T_{winter}	0.0065	0.0036	1.776	0.0853	−195.31
	<i>F</i>	df	<i>p</i>	Adjusted <i>R</i> ²	
Model for $\delta^{15}\text{N}$	5.37	29	0.0013	0.39	−80.958
Multiple regression terms	Estimate	SE	<i>t</i>	<i>p</i>	
$P_{\text{prevSummer}}$	−0.0080	0.0033	−2.422	0.0219	−76.51
SNM_{prev}	−0.0104	0.0048	−2.162	0.0390	−77.73
$S_{\text{prevSummer}}$	−0.1206	0.0517	−2.333	0.0268	−76.94
P_{spring}	0.0037	0.0018	2.078	0.0467	−78.10
$T_{\text{prevSummer}}$	0.1309	0.0635	2.061	0.0484	−78.18

west. Also Virkkula *et al.* (2003) determined that the main sources of NO_x in Sevetijärvi approximately 200 km north of Värriö are the regions of heavy traffic in southern Finland and continental Europe. The origin of N deposited is thus possibly fossil fuel-related and depleted in ^{15}N (Hastings *et al.* 2009). The depleted isotopic value of deposited N is reflected in the $\delta^{15}\text{N}\text{‰}$ values at this site. There was no trend in the two other $\delta^{15}\text{N}\text{‰}$ time series. The reasons why no trends were found for the other sites could be the opposite effects of source $\delta^{15}\text{N}$ from deposition and increased soil N availability due to deposition and slight warming trend over the study period.

Our and other studies' results suggest that sites having higher N% also have higher $\delta^{15}\text{N}\text{‰}$. However, the annual resolution in our time series revealed that this does not apply to the variations among years. N% correlated systematically negatively with temperatures, whereas $\delta^{15}\text{N}\text{‰}$ correlated positively. The strongest relationships were found between previous growth-period temperatures and $\delta^{15}\text{N}\text{‰}$ and N% time series. Despite attempts, we did not find a single environmental factor or a simple combination of factors that affect the N composition or concentration (Fig. 7).

Our results suggest that in the short term, the same factors that potentially increase growth also lower the N% in berries. In earlier studies it was found that high temperatures during the previous summer promote vegetative growth of *E. nigrum* (Shevtsova *et al.* 1995, Shevtsova *et al.* 1997). Similarly, warming experiments conducted in the arctic showed consistent increases in plant biomass as a reaction to warming (Dormann and Woodin 2002, Walker *et al.* 2006). Contrary to biomass, and in line with our findings, most earlier studies report warming to be associated with reduced N concentrations in plant tissues (Dormann and Woodin 2002, Aerts *et al.* 2009; see however Welker *et al.* 2005). The reduction in N was explained by dilution of N in increased leaf mass. Dormann and Woodin (2002) suggested that decreases in the leaf nitrogen concentration occur because the effect of increased temperature on growth is stronger than the effect of increased N uptake due to higher soil N availability. Our measurements, in contrast to other studies mentioned here, were made on seeds. In many plant

species favourable conditions and even moderate increases in N availability can increase seed starch content, which decreases the concentration of seed proteins (Wang *et al.* 2008). However, it also seems that in favourable conditions arctic species invest first in vegetative growth and then in reproduction (Arft *et al.* 1999). It is possible that good growth conditions and thus reduced N in seeds is a consequence of allocation of protein reserves into growth rather than into reproduction.

The N isotopic composition in plants is not affected by changes in allocation or dilution but by the availability and the source of N. The best determinants of $\delta^{15}\text{N}\text{‰}$ in crowberry seeds according to our results were previous summer growth conditions and snowmelt, though the model had low explaining power. Based on this and divergent correlations between previous summer temperatures and $\delta^{15}\text{N}\text{‰}$ and N% we suggest that the process behind these relationships is temperature-increased mineralization of nutrients that increases available N or changes the source of N to the crowberry, seen as changes in $\delta^{15}\text{N}\text{‰}$. The change in temperature and N availability at the same time triggers growth, which is seen as lowered N% in berries in the short term. However, in the long run increasing temperature and deposition may increase N concentrations in addition to $\delta^{15}\text{N}$.

Berry production in ericoid species is known to be affected by climate conditions both in current and previous year. Good yields require favorable conditions during bud formation, flowering in spring and ripening of fruits in summer (Kuchko 1988). In our study area, flowering of the crowberry takes place in May and June. Our results imply that multiple factors do effect the berry production and that most of these factors actually occur already in the previous year. Especially, our analysis suggests that the *E. nigrum* berry production is negatively related to autumn temperatures in the previous year. This is somewhat similar to findings of Selås (2000), who reported preceding August temperatures to have a negative relationship with the *Vaccinium myrtillus* berry production in a 50-year time series from southern Norway. Snow cover in the past winter that protects the shrubs from low temperatures has been raised in many studies as an important factor for both growth and repro-

duction of ericoid species (e.g. Raatikainen and Vänninen 1988, Selås 2000, Aerts 2010, but see Olofsson *et al.* 2011), however, our results did not support this. This is probably because in our study area snow depth was always sufficient to protect the shrubs against freezing.

Conclusions

We measured $\text{N}\%$ and $\delta^{15}\text{N}\%$ in crowberry seeds in a subarctic environment. Higher N concentrations and higher $\delta^{15}\text{N}$ values were found in crowberry seeds from the forested site than in seeds from higher altitude fells where there are few trees; and we detected an increasing trends in $\text{N}\%$ and $\delta^{15}\text{N}\%$ during the last 35 years at the forested site. However, there was a contrasting decreasing trend in $\delta^{15}\text{N}\%$ at higher elevations on the fell. This was seen as an indication of an increase in plant available nitrogen at the forested site and an evidence for the role of proportionately more depositional N with different isotopic composition on the fell. At an annual scale, the variations in $\text{N}\%$ and $\delta^{15}\text{N}\%$ were mainly dependent on the previous summer or the previous growth-period temperatures, however, the effect was in opposite directions for $\text{N}\%$ and $\delta^{15}\text{N}\%$. The lowered N concentrations that were found in berries a year after a warm summer were interpreted as changes in allocation when the plant invests in rapid growth. Increased $\delta^{15}\text{N}\%$ was interpreted as an indication of increased N availability or changes in the source of N to the crowberry. The changes in berry production among years were dependent on multiple factors, mainly the environmental conditions of the previous year.

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